The Spleen in Type I Hyperlipoproteinemia

Histochemical, Biochemical, Microfluorometric and Electron Microscopic Observations

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Histochemical, biochemical, microfluorometric and electron microscopic studies were made of the spleen of a patient with type I hyperlipoproteinemia. Foam cells were observed that contained a material identified as ceroid on the basis of its autofluorescence, acid-fastness, sudanophilia, PAS-positivity and insolubility in organic solvents. Electron microscopy showed that the ceroid was organized in the form of granules with concentric lamellae of irregular periodicity. The process of formation of these granules is described in detail. The ceroid was considered to represent nondigestible end products of the metabolism of chylomicrons taken up by macrophages in splenic sinusoids (Amer J Path 64:67–96, 1971).

TYPE I HYPERLIPOPROTEINEMIA (familial hyperchylomicronemia, Bürger-Grütz syndrome) is characterized by the presence of large numbers of chylomicrons in plasma 14 hours or longer after ingestion of the last meal of a normal diet; chylomicrons disappear from plasma within a few days after a fat-free diet is instituted. The underlying biochemical lesion is believed to be a deficiency in the activity of the tissue lipase having particular affinity for triglycerides in lipoproteins.[1,2] At the present time, this deficiency is determined only indirectly by measurements of lipolytic activity occurring transiently in plasma after administration of heparin.[3,4] Since triglyceride hydrolysis in plasma is not considered to be an important process in the normal removal of chylomicrons, it is believed that triglycerides are partially or completely hydrolyzed within capillary endothelial cells, in which lipoprotein lipase activity has been demonstrated.[5] In type I hyperlipoproteinemia, a deficiency of lipoprotein lipase is presumed to exist in these cells and in other tissue sites involved in the removal of triglycerides present in chylomicrons and other lipoproteins. In this disease, therefore, chylomicrons remain circulating in plasma for long periods of time and are removed by other mechanisms that normally

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may be inoperative or only of minor importance. Dependence on these secondary processes of chylomicron removal places severe limitations on the rates of disposal of lipids. A consequence of such limitations is the formation of foam cells, which have been described [1,2,6] in spleen, liver, lymph nodes and bone marrow of patients with type I hyperlipoproteinemia. Little is known of the pathologic anatomy of this disorder, and no histochemical or electron microscopic studies have been made of the foam cells present in patients with type I hyperlipoproteinemia.

The present report describes histologic, histochemical, biochemical and electron microscopic characteristics of the spleen in a patient with type I hyperlipoproteinemia. Emphasis is placed on the features of the foam cells, which are compared with those in other lipidoses, and an attempt is made to explore the mechanisms by which chylomicrons are metabolized in type I hyperlipoproteinemia.

Report of Patient

LW (CC No. 03-63–94), a 27-year-old man, whose clinical and family history was reported in detail in 1966,[1] developed eruptive xanthomas, lipemia retinalis, hyperchylomicronemia and hepatosplenomegaly in early infancy, and bouts of abdominal pain beginning at 7 years of age. The diagnosis of type I hyperlipoproteinemia was confirmed on many occasions by analysis of plasma lipids before and after restriction of dietary fat intake. Plasma levels of post-heparin lipolytic activity in this patient were normal in at least 30 analyses by the Ediol method [3] over a 9-year period; however, more specific analyses in 1969 showed that the patient had normal activity of plasma post-heparin monoglyceride hydrolase and very low triglyceride lipase activity.[4] Bone marrow aspiration at age 25 disclosed the presence of foam cells. The episodes of abdominal pain gradually increased in frequency and severity, requiring 4 hospitalizations between September 1968 and March 1969. A radioisotope scan with ^{99m}Tc sulfide colloid was performed on March 24, 1961, and revealed an area of decreased uptake in the medial aspect of the spleen. An arteriogram, obtained by selective catheterization of the splenic artery on March 25, 1969, showed several areas of reduced to absent flow during the splenogram phase of the study. The diagnoses of recurrent splenic infarction and chronic pancreatitis were made. The patient improved transiently on symptomatic treatment and a fat-free diet; however, on May 24, 1969, he was again hospitalized because of left-sided abdominal pain of 10 days' duration. The spleen was removed on June 5, 1969. A mass of

fibrous and inflammatory tissue was found in the tail of the pancreas and was considered to represent evidence of chronic pancreatitis.

The patient's plasma lipids, while on a regular American diet and after a week on a diet consisting of less than 5% fat, have been reported previously.[1] The respective values while on each diet were: triglycerides, 4200 and 220 mg/100 ml; total cholesterol, 360 and 135 mg/100 ml (of which 47 and 66%, respectively, were esterified); and phospholipids, 454 and 184 mg/100 ml. The values on the fat-free diet approximated those obtained for several days prior to splenectomy. Because dietary control was erratic, the mean triglyceride concentrations between hospitalizations are unknown, but probably ranged between 1000 and 2000 mg/100 ml.

The spleen weighed 510 g and measured 15 × 11 × 4 cm. Microscopic study showed good preservation of follicular architecture, moderate congestion of the red pulp and sinusoids, and diffuse, moderate infiltration of the red pulp by polymorphonuclear leukocytes. Small groups of large (20 to 60 μ in diameter) histiocytes with abundant, pale foamy cytoplasm and distinct borders were present throughout the red pulp (Fig 1 and 2).

Results

Histochemical Observations

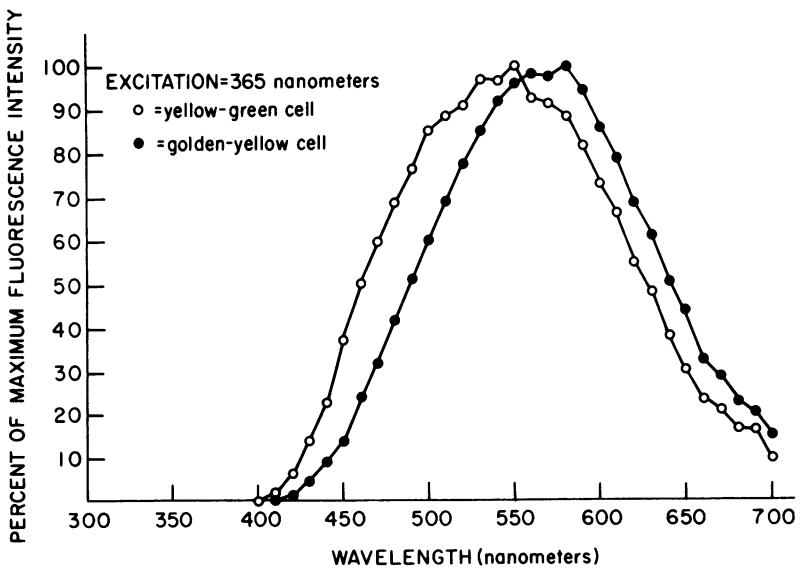
The foamy appearance of the splenic histiocytes in sections stained with hematoxylin-eosin was due to the presence of spherical, nonbirefringent granules (Fig 3) that measured up to 3 μ in diameter and stained with oil red O, Sudan black B (Fig 4 and 5), the Baker method for phospholipids, the Schultz method for cholesterol (Fig 6), the Ziehl-Neelsen method for acid-fast substances and the Nile blue method of Lillie for lipofuscin.[7] These granules also were stained by the periodic acid-Schiff (PAS) method before and after treatment of sections with amylase for the removal of glycogen (Fig 7). The PAS-positive material was largely removed from unfixed frozen sections by extraction with chloroform-methanol; however, extraction of either frozen or paraffin sections of formalin-fixed tissue failed to remove the PAS-positive material. Acid-fastness and sudanophilia of the granules were demonstrated also in paraffin sections. The granules were non-metachromatic and stained weakly with the colloidal iron and Alcian blue methods for acid carbohydrates. They showed a reaction with esterase [8] and acid phosphatase [9] methods (Fig 8 and 9), but not with the adenosine triphosphatase,[10] thiamine pyrophosphatase,[11] nucleoside diphosphatase [11] and

alkaline phosphatase [12] technics. Small granules that also stained with PAS, Sudan black B, Nile blue, esterase (Fig 8) and acid phosphatase (Fig 9) methods were present in many cells in the lining of splenic sinusoids.

Study of frozen sections stained with benzpyrene [13] for the fluorescence microscopy of lipids showed that the walls of blood vessels throughout the spleen were heavily and diffusely infiltrated with small lipid droplets (Fig 10 and 11), which showed no autofluorescence in unstained sections. These droplets stained also with Sudan black B and oil red O, but not with the other technics mentioned above.

Microfluorometric Observations

Examination of unstained tissue sections by fluorescence microscopy revealed the presence of intense autofluorescence in the granules of foam cells and sinusoidal lining cells (Fig 12 and 13). The color of this fluorescence varied from yellow-green to golden-yellow. The spectral distribution of this fluorescence was measured in individual cells with a Zeiss microspectrofluorimeter. The data obtained were corrected according to the spectral sensitivity curve of the photomultiplier tube and are shown in Text-fig 1. Cells with a yellow-green fluorescence



TEXT-FIG 1—Fluorescence emission spectra of ceroid-laden splenic macrophages in type I hyperlipoproteinemia, showing variations in the spectral distribution of the fluorescence in individual cells.

had a fluorescence emission maximum at 550 nanometers (nm) and a smaller peak at 530 nm, while cells with a golden-yellow fluorescence had an emission maximum at 580 nm and a smaller peak at 560 nm.

Biochemical Observations

The lipid content of the spleen was analyzed quantitatively according to methods described previously.[14] Total phospholipids were 10.95 mg/g of wet tissue (normal range 12.8 to 17.6 mg/g[15]); total cholesterol, 4.77 mg/g of wet tissue (normal range 4.6 to 5.8 mg/g[15]), and total triglycerides, 2.53 mg/g of wet tissue (normal, less than 0.4 mg/g[15]). Qualitative, one-dimensional thin-layer chromatograms of lipid extracts revealed a decrease in lecithin; sphingomyelin and glycolipid spots appeared within normal limits.

Electron Microscopic Observations

For electron microscopy, tissues were fixed for 2 hours with cold 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2; washed with several changes of 5% sucrose in 0.1 M phosphate buffer, pH 7.2; post-fixed for 2 hours with cold 1% osmium tetroxide in Millonig's phosphate buffer; dehydrated in a graded series of alcohols and propylene oxide, and embedded in Maraglas.[16] Semithin (0.5 to 1 μ thick) sections of Maraglas-embedded tissues were stained with alkaline toluidine blue and examined by light microscopy for purposes of locating the foam cells. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with an RCA EMU 3G electron microscope.

Mature Foam Cells. Electron microscopic study showed that the granules in foam cells (Fig 14–17) were intensely osmiophilic and often surrounded by single limiting membranes. Their size and morphology varied considerably (Fig 14 and 15) even within single cells. The entire content of some granules was arranged in the form of concentric lamellae of variable periodicity (40 to 100 Å) (Fig 16 and 17). Other granules contained one or more groups of closely spaced (40 Å) lamellae and less electron-dense areas that were formed either by widely spaced lamellae, some of which occurred in pairs, or by amorphous material. Clear, cleft-like spaces often were present in the granules.

The cells containing the granules were round or oval (Fig 14 and 15). Their plasma membranes were smooth with few infoldings or pinocytotic vesicles. The nuclei were usually eccentrically located. The nucleoli were prominent and the chromatin was marginated against

the inner aspect of the nuclear membranes. Few mitochondria were present; they were small and contained sparse cristae and a clear matrix. The granular endoplasmic reticulum was composed of small numbers of slightly dilated cisterns. Agranular reticulum and Golgi elements were poorly developed and inconspicuous. Fine filaments, 30 to 50 Å in diameter, were scattered throughout the cytoplasm.

Developing Foam Cells. Different stages of formation of mature granules were identified (Fig 18–26) in reticulum cells and young histiocytes in the cords of Billroth and in the lining of the splenic sinusoids. The distribution of these cells corresponded to that of cells in which small, autofluorescent granules were identified by light microscopy. These cells were stellate-shaped, with highly developed cytoplasmic processes that extended between adjacent lining cells and into the lumens of splenic sinusoids. They contained single nuclei with marginated chromatin, moderate numbers of mitochondria, variable amounts of granular endoplasmic reticulum and Golgi elements, and numerous fine cytoplasmic filaments of the type present in cells with fully formed granules. Some of these cells also contained whole and fragmented erythrocytes in various stages of degradation; less frequently, they contained ingested platelets and leukocytes. The young histiocytes described above had only few large cytoplasmic granules of the type found in the foam cells. Instead, they contained variable numbers of smaller granules, 1000 to 4000 Å in diameter, and arrays of branching and convoluted tubules and cisterns that measured from 500 to 1200 Å in diameter (Fig 18–21). These structures were usually concentrated in the cytoplasmic processes of these cells. They were limited by osmiophilic, single, trilaminar membranes and contained a finely granular, moderately electron-dense material, peripheral areas of which had a zone of decreased electron density that measured from 70 to 150 Å in width (Fig 20, 21, 23 and 24). Electron-dense, concentric lamellae were frequently observed adjacent to this peripheral zone of decreased electron density in the granules and in knob-like dilatations at the ends of tubules and cisterns (Fig 21, 23 and 24). These lamellae measured from 20 to 25 Å in thickness and often formed pairs. The two components of each pair were separated from each other by a space of 40 to 50 Å, and from adjacent pairs by spaces of 55 to 100 Å. In their narrowest regions, the tubules contained two electron-dense lamellae that measured 20 to 25 Å in thickness, were separated from each other by spaces of 40 to 45 Å, and also were surrounded by a peripheral zone of decreased electron density (Fig 21).

Smaller, poorly oriented, developing lamellae were sometimes discernible in the electron-dense, finely granular matrix of the wider tubules and cisterns (Fig 21).

The plasma membranes of young histiocytes were highly convoluted and often had numerous pinocytotic vesicles and coated vesicles as well as deep, thin-walled, tubular and slit-like invaginations that extended into the cell depth (Fig 18 and 22–25). Such invaginations contained a material that was less electron-dense (Fig 18) than that undergoing organization in the thicker-walled tubules, cisterns and vesicles described above. Very electron-dense material was occasionally found in tubules open to the cell surface (Fig 22). No direct continuity could be demonstrated between the cell surfaces and areas that contained organized lamellae; however, some vesicles present in the vicinity of the plasma membranes (Fig 24) showed continuity with elements of granular endoplasmic reticulum (Fig 25). These vesicles contained moderately electron-dense material. The areas of reticulum with which they made contact were dilated, had a denser content than the remainder of the reticulum and were partially denuded of ribosomes (Fig 25). Similarly altered elements of granular reticulum were continuous in some area with the tubules and cisterns that contained denser and more organized material (Fig 18 and 26).

Cells that contained few electron-dense granules, tubules and cisterns were rich in Golgi components and the usual type of granular and agranular reticulum (Fig 27). These organelles decreased considerably in number as the quantity of electron-dense granules and associated structures became progressively greater (Fig 18 and 19).

Lipid droplets that ranged from 0.01 to 0.2 μ in size were found in the basement membranes of blood vessels of various sizes (Fig 28). Intracellular accumulation of lipid in vascular endothelium was observed rarely.

Ultrastructural Observations on the Localization of Acid Phosphatase Activity

For the ultrastructural localization of acid phosphatase activity, glutaraldehyde-fixed tissues were sectioned at a thickness of 75 μ with a Smith-Farquhar tissue sectioner; incubated at pH 5.0 with cytidine-5′-monophosphate; washed with several changes of buffer; postfixed with osmium tetroxide and prepared for electron microscopy.[17] As shown in Fig 29 and 30, the heaviest deposition of reaction product occurred in the developing, electron-dense tubules and cisterns of the younger histiocytes; the larger, more mature granules showed a less intense reaction, usually in their periphery.

Discussion

The following pathologic changes were observed in the spleen of our patient with type I hyperlipoproteinemia: deposition of lipid droplets in the walls of blood vessels; congestion of the red pulp; infiltration of the red pulp by moderate numbers of polymorphonuclear leukocytes and the presence of foam cells that contained large numbers of autofluorescent, acid-fast, sudanophilic, and PAS-, Baker- and Schultz-positive granules. The splenomegaly resulted from congestion rather than from infiltration by foam cells, which were not present in sufficient numbers to account for the splenic enlargement. The etiology of the congestion and inflammatory reaction is unclear; these changes may have been related to pancreatitis.

The lipid droplets in the vascular walls probably represented chylomicrons that had passed through endothelium and had become trapped in the basement membranes of blood vessels. The mechanisms by which chylomicrons cross the endothelial barrier have been studied by Parker and Odland [18] and by Dobbins and Rollins,[19] who suggested that they pass by vesicular transport through the cytoplasm of endothelial cells rather than directly through their intercellular junctions. The leakage of chylomicrons from the vascular compartment is evidently responsible for the accumulation of foam cells in perivascular areas.

Nature of the Content of Foam Cells in Type I Hyperlipoproteinemia

The foam cells in type I hyperlipoproteinemia are macrophages, as shown by their high content of acid phosphatase and esterase. The material in the granules of these cells is ceroid, as shown by its sudanophilia, acid-fastness, autofluorescence and insolubility in organic solvents, which are all properties characteristic of ceroid.[20–22] Previous measurements of the fluorescence emission spectrum of ceroid in other tissues have shown a peak at 490–510 nm according to Hartroft and Porta,[22] and two peaks, at 440–460 and 530–560 nm, according to Hydén and Lindstrom.[23] Hartroft and Porta [22] observed that the visual appearance of the fluorescence of experimentally produced ceroid changed from yellowish-white to golden yellow as the stages of ceroid formation progressed. Neglia *et al* [24] studied lipofuscin produced experimentally by intravenously administered fat emulsions and also observed a gradual change in the color of the fluorescence from yellow-green to golden-brown. We found variations from 550 to 580 nm in the spectral peaks of different ceroid-laden cells in the spleen. These variations probably represented differences either in the chemical composition or stage of formation of the granules.

Ceroid, one of the lipid pigments of the lipofuscin group, is a product of the oxidation and polymerization of fatty acids and phospholipids.[21,22] Lipofuscin pigments occur normally in most tissues, including spleen.[21,22] Deposition of ceroid in various tissues has been observed in vitamin E deficiency, malabsorption, ceroid storage disease, β-lipoprotein deficiency, and experimental dietary cirrhosis.[21] Variable amounts of ceroid have been found in association with deposits of other lipids in atheromatous plaques, areas of fat necrosis, xanthomas, eosinophilic granulomas, several types of neoplastic cells, in neurons of some patients with amaurotic idiocy[21] and in various tissues of animals given fat emulsions intravenously. The preceding evidence supports the concept that ceroid can develop, as a secondary change, from the oxidation and polymerization of lipids stored in various disorders, especially in older patients.[21] Therefore, the presence of ceroid cannot be considered a specific diagnostic feature. Ceroid-containing foam cells, similar to those in type I hyperlipoproteinemia, also have been found in the spleen in ceroid storage disease[25-28] and in type III hyperlipoproteinemia.[29] The etiology of ceroid storage disease is unclear, and it is uncertain that patients reported with ceroid storage disease or lipofuscinosis represent instances of a single, specific disease entity. Winkler *et al*[28] studied a patient with ceroid storage disease who had a history of prolonged intake of a diet that contained large amounts of polyunsaturated fatty acids and cystine. This diet was similar to that used to produce ceroid deposition and hepatic cirrhosis in experimental animals.[30] Winkler *et al* postulated that such a diet may have led to a state of relative vitamin E deficiency with subsequent formation of ceroid from polyunsaturated fatty acids. Vitamin E is an antioxidant that prevents the peroxidation of unsaturated fatty acids, for which reason experimental vitamin E deficiency results in the formation of large amounts of ceroid.[21,22] The deposition of ceroid in type I hyperlipoproteinemia may be related also to a state of relative deficiency of vitamin E, increased amounts of which may be required for the metabolism of the abnormally large quantities of fatty acids ingested by macrophages in the form of triglyceride-rich chylomicrons.

No data are available on the ultrastructure of splenic foam cells in ceroid storage disease. The pleomorphic nature of the lipofuscin group of pigments is well known,[22] and poorly organized lamellae have been observed in lipofuscin granules in normal spleen[31,32] and other tissues,[22] in ceroid deposits in human atheroma[33] and in the ceroid-containing histiocytes in colonic histiocytosis.[34] The lipofuscin granules in animals given intravenous fat emulsions did not contain organized lamellae.[24] Although macrophages in normal spleen contain autofluorescent gran-

ules,[35] the large foam cells described in this study have not been observed in previous studies of normal spleen.[36–39]

Characteristics of Foam Cells in Other Types of Hyperliproteinemia

Foam cells have been observed by light microscopy in spleens of patients with uncontrolled diabetes mellitus and hyperlipemia of undetermined type.[40] Recent evidence, reviewed below, shows that foam cells with various histochemical and ultrastructural similarities to those in type I hyperlipoproteinemia are present in patients with type III and type V hyperlipoproteinemia. The limited light microscopic data available on foam cells in the spleen of patients with well-defined types of hyperlipoproteinemias were summarized by Roberts *et al*,[29] who did not find such cells in 6 patients with type II hyperlipoproteinemia or in 7 patients with type IV hyperlipoproteinemia; however, in a patient with type III hyperlipoproteinemia, Roberts *et al* observed large, autofluorescent foam cells that were histochemically similar to those in the patient with type I hyperlipoproteinemia. We have restudied the spleen in the patients with types II and IV hyperlipoproteinemia reported by Roberts *et al*[29] and found only a few, small, scattered, weakly autofluorescent cells. These cells could not be recognized as foam cells in preparations stained with hematoxylin-eosin, and were similar to those in patients who had normal lipoprotein patterns.

Tanaka *et al*[41] described the ultrastructure of foam cells in the bone marrow of a patient with hyperlipoproteinemia now known to be nonfamilial type V. These cells contained numerous lipid droplets and granules. Some granules were composed of concentric lamellae with a periodicity of 100 Å and were similar to those in the foam cells in the spleen of the patient with type I hyperlipoproteinemia; other granules had large, empty spaces that represented lipid material that had dissolved during tissue preparation. In contrast to the foam cells in type I hyperlipoproteinemia, those in type V showed strong birefringence, probably due to their high content of cholesterol. Different stages in the formation of foam cells were not observed, and no studies were made to determine whether or not these cells were autofluorescent or acid-fast. However, we have made further study of the bone marrow of the patient with type V hyperlipoproteinemia reported by Tanaka *et al*[41] and have found no autofluorescence in the foam cells.

Differentiation of Foam Cells in Type I Hyperlipoproteinemia from Those in Other Lipidoses.

The characteristic autofluorescence and acid-fastness of ceroid serve to differentiate this material from those found in foam cells in a num-

ber of other lipidoses unrelated to abnormalities of plasma lipoproteins. Diezel [42] has described other types of autofluorescence in lipid deposits in storage cells in the central nervous system in Tay-Sachs disease (blue-green), gargoylism (light blue) and metachromatic leukodystrophy (blue-green); however, no spectral measurements have been made of these types of fluorescence, which appear visually different from that of ceroid. In our experience, the deposits in Gaucher's disease, Fabry's disease and GM_1 gangliosidosis do not show autofluorescence.

The lack of birefringence differentiates the foam cells in type I from those in type V hyperlipoproteinemia,[41] Fabry's disease,[43] Wolman's disease [44] and Tangier disease,[45] in which the lipid deposits are birefringent. Other histochemical properties of the material in type I hyperlipoproteinemia, such as its reaction with the PAS, Sudan and benzpyrene technics, are similar to those of other, biochemically different lipid deposits. Glycolipids in Gaucher's disease,[46] Fabry's disease [43] and the various types of gangliosidosis [47] are PAS-positive, and the Sudan and benzyprene methods stain most lipids regardless of their chemical composition. However, the foam cells in type I hyperlipoproteinemia are ultrastructurally different from those in the following: Gaucher's disease [46] in which the foam cells contain tubules rather than granules with concentric lamellae; GM_1 gangliosidosis,[48] in which they contain tubules smaller than those in Gaucher's disease; Fabry's disease [43] in which the concentric lamellae exhibit a much more regular, well-defined periodicity, and Wolman's disease [49] in which large cholesterol clefts are present in the cells.

Ultrastructural differentiation of foam cells in type I hyperlipoproteinemia from those in Niemann-Pick's disease [41,50] is difficult because of the marked similarities in the appearance of the granules in these two diseases. The histochemical criteria, discussed above, may provide a more reliable basis for a morphologic differentiation of these two diseases. It should be mentioned however, that only limited histochemical and electron microscopic data are available on the various types of Niemann-Pick's disease described recently,[51] and that we have found considerable amounts of ceroid in splenic foam cells of an 11-year-old patient with biochemically proven type D of Niemann-Pick's disease.

Morphogenesis of Foam Cells in Type I Hyperlipoproteinemia

The location of foam cells in the spleen, bone marrow and lymph nodes of patients with type I hyperlipoproteinemia reflects the fact that the presence of macrophages in the lining of sinusoids in these organs gives these cells direct access to the abnormally large numbers of circulating chylomicrons in this disorder.

The phagocytic properties of macrophages are well known, and several studies have shown that the uptake of large particles by reticuloendothelial cells is mediated by tubular infoldings of their plasma membranes. Törö *et al* [52] showed that the uptake of India ink particles by Kupffer cells occurred by infoldings that formed worm-like, elongated, frequently branching structures, which were found in most cases under the cell surface and often were connected with it. These structures measured from 1000 to 1500 Å in width, were filled with a material of moderate electron density and had a denser line in their central areas. In later stages of uptake, the tubules disappeared and the India ink particles were found in large round vacuoles, which were presumed to be derived from the tubular structures. Similar images of phagocytosis were described in Kupffer cells by Orci *et al* [53] and Matter *et al* [54] and in splenic macrophages by Orci *et al.*[53] According to Matter *et al*,[54] no connections with the endoplasmic reticulum were observed in these structures. Such a process, which Orci *et al* called "micropinocytosis vermiformis," was stimulated by partial hepatectomy and by the administration of tetracycline or streptozotocine.[53] Matter *et al* [54] also described the association of numerous coated vesicles with these tubules. They postulated that colloidal particles are first fixed to the surface of the cell, after which they are transported toward the interior of the cell by membrane flow, and that the coated vesicles mediate the transport across the plasma membrane into the cytoplasm. Burke and Simon [55] also observed tubular invaginations of the plasma membranes of splenic macrophages in association with the uptake of colloidal carbon, but did not describe the dense central line found in such tubules by others.[52–54] Burke and Simon [55] emphasized that many vacuoles formed by infoldings of the plasma membranes were tubular in appearance, creating the impression that the ingested carbon particles were in dilated elements of smooth endoplasmic reticulum.

Only limited information is available on morphologic details of the uptake of chylomicrons by reticuloendothelial cells. Ashworth *et al* [56] showed that Kupffer cells take up lipid droplets when exposed to chylomicron-rich blood. Neglia *et al* [24] observed the development of large numbers of lipofuscin granules in Kupffer cells and hepatic parenchymal cells of animals given multiple intravenous infusions of fat emulsions. These workers concluded that such lipofuscin granules contained the end products of the lysosomal digestion of lipid droplets, which entered the cells by pinocytosis and were surrounded by single membranes pinched off from the plasma membranes at the time of uptake. A similar mechanism of chylomicron uptake by endothelial cells was proposed by Dobbins and Rollins [19] in their study of

intestinal lymphatics. None of these investigators, however, described an association between tubular invaginations of the plasma membranes and uptake of chylomicrons. Neglia *et al*[24] in their relatively long-term (105 days) study of the effects of intravenous fat emulsions did not observe in Kupffer cells the extensive network of tubules and cisterns with electron-dense content that we found in splenic macrophages of our patient with type I hyperlipoproteinemia.

The small, thin-walled tubules that were observed in this study in association with infoldings of the plasma membranes of macrophages did not have the central line described by others [52–54] in studies of the uptake of other particulate materials. Instead, these structures contained a moderately electron-dense material, which we believe represents chylomicron-derived lipid in the process of uptake. These observations suggest that chylomicrons were at least partially fragmented or otherwise altered in shape upon uptake, since the diameter of these tubules was smaller than that of intact chylomicrons. We did not observe the early phases of actual engulfing of chylomicrons. We attribute this to the fact that the spleen was removed at a time when, due to restriction of dietary fat intake, our patient did not have hyperchylomicronemia.

The next morphologically recognizable stage in the metabolism of chylomicron-derived material was the formation of thick-walled, electron-dense, acid phosphatase-positive tubules, cisterns and vesicles. The exact process by which such structures were formed is unclear. Continuity was demonstrated between the endoplasmic reticulum and the thin-walled vesicles and tubules, as well as between the endoplasmic reticulum and the thicker-walled, more electron-dense structures. It is possible that the thin-walled structures acquired their acid phosphatase upon fusion with the endoplasmic reticulum, after which they were transformed directly into the thicker-walled tubules and cisterns. It is also possible that the thin-walled tubules and vesicles may have discharged their content into the endoplasmic reticulum, where this material may have been partially metabolized. Then the electron-dense, thicker-walled tubules and cisterns may have formed *de novo* from the segregation of non-metabolized lipid components in areas of endoplasmic reticulum that were different and separate from those that fused with the thinner-walled structures derived from plasma membranes. In either case, the presence of acid phosphatase in the thicker-walled structures indicates that chylomicron-derived material became segregated into structures that had the characteristics of lysosomes, where this material gradually was transformed into ceroid granules composed of concentric lamellae.

The continuity found in this study between granular endoplasmic

reticulum and lysosomal structures is reminiscent of that which we have observed previously in Gaucher cells.[46] In Gaucher cells, acid phosphatase-positive lysosomes laden with developing tubules were shown to be continuous with segments of granular endoplasmic reticulum that were partially denuded of ribosomes. The possible role of the Golgi apparatus in this process is uncertain. The Golgi apparatus plays a part in the biosynthesis and secretion of lipoproteins.[57] It is possible that the Golgi apparatus participated in the catabolism of chylomicrons in the splenic macrophages of our patient by providing primary lysosomes that may have fused with, and transferred their hydrolytic enzymes to, lipid-containing structures derived from the plasma membranes. Such a mechanism, however, was not observed in our study. It is more likely, therefore, that the lysosomal enzymes were derived from the endoplasmic reticulum by one of the mechanisms proposed above.

The presence of a variety of lipolytic enzymes in lysosomes and the role of the endoplasmic reticulum in lipid metabolism are well known. These facts and the morphologic and biochemical evidence presented in this study provide a basis for the concept that the splenic foam cells in type I hyperlipoproteinemia are macrophages laden with residual bodies that contain the end products of the metabolism of chylomicrons by the endoplasmic reticulum and lysosomes. Nevertheless, it should be pointed out that type I hyperlipoproteinemia is not a true "lysosomal disease,"[58] even though lysosomes are the sites of storage of the accumulated lipids. This disease is not due to a deficiency of a lysosomal enzyme, and the excessive intralysosomal accumulation of ceroid derived from lipid occurs as an abnormality secondary to a deficiency in lipoprotein lapase, a nonlysosomal enzyme [5] with an alkaline pH optimum.[4] These features of type I hyperlipoproteinemia contrast with those of Wolman's disease, a clinically different entity in which large amounts of cholesterol esters and triglycerides accumulate in foam cells as the result of a deficiency in a lysosomal acid lipase.[59,60]

It is not known to what extent some of the observations made in this study represent part of the normal processes of chylomicron metabolism. It is clear that the accumulation of ceroid is quantitatively abnormal; however, the functional significance of this phenomenon is unknown.

References

1. Fredrickson DS, Lees RS: Familial hyperlipoproteinemia, The Metabolic Basis of Inherited Disease. Edited by JB Stanbury, JB Wyngaarden, DS Fredrickson. New York, McGraw-Hill Book Company, 1966, pp 429–485

2. Fredrickson DS, Levy RI, Lees RS: Fat transport in lipoproteins: an integrated approach to mechanisms and disorders. New Eng J Med 276:32–44, 94–103, 148–156, 215–225, 273–281, 1967
3. Fredrickson DA, Ono K, Davis LL: Lipolytic activity of post-heparin plasma in hypertriglyceridemia. J Lipid Res 4:24–33, 1963
4. Greten H, Levy RI, Fredrickson DS: Evidence for separate monoglyceride hydrolase and triglyceride lipase in post-heparin human plasma. J Lipid Res 10:326–330, 1969
5. Blanchette-Meckie EJ, Scow RO: Sites of lipoprotein lipase activity in perfused adipose tissue: an electron microscopic cytochemical study. J Cell Biol (in press)
6. Hudson P, Moon JH: Clinicopathological conference. Virginia Med Monthly 92:321–328, 1965
7. Bowling MC: Histopathology Laboratory Procedures of the Pathologic Anatomy Branch of the National Cancer Institute. Washington, DC, US Government Printing Office, 1967
8. Barka T, Anderson PJ: Histochemistry: Theory, Practice and Bibliography. New York, Harper & Row, Publishers, 1963, pp 257–277
9. *Idem*:[8] pp 240–241
10. Rostgaard J, Behnke O: Fine structural localization of adenine nucleoside phosphatase activity in the sarcoplasmic reticulum and T system of rat myocardium. J Ultrastruct Res 12:579–591, 1965
11. Novikoff AB, Goldfischer S: Nucleoside diphosphatase activity in the Golgi apparatus and its usefulness for cytological studies. Proc Nat Acad Sci 47:802–810, 1961
12. Burstone MS: Enzyme Histochemistry and its Application to the Study of Neoplasms. New York, Academic Press, Inc, 1962, pp 275–276
13. Berg NO: A histological study of masked lipids. Acta Path Microbiol Scand: Suppl 90, 1951
14. Kwiterovich PO, Sloan HR, Fredrickson DS: Glycolipids and other lipid constituents of normal human liver. J Lipid Res 11:322–330, 1970
15. Sloan HR, Fredrickson DS: Unpublished data.
16. Freeman J, Spurlock BO: A new epoxy embedment for electron microscopy. J Cell Biol 13:437–443, 1962
17. Ferrans VJ, Hibbs RG, Buja LM: Nucleoside phosphatase activity in atrial and ventricular myocardium of the rat: a light and electron microscopic study. Amer J Anat 125:47–86, 1969
18. Parker F, Odland G: Electron microscopic similarities between experimental xanthomas and human eruptive xanthomas. J Invest Derm 52:136–147, 1969
19. Dobbins WO, Rollins EL: Intestinal mucosal lymphatic permeability: an electron microscopic study of endothelial vesicles and cell junctions. J Ultrastruct Res 33:29–59, 1970
20. Endicott KM, Lillie RD: Ceroid, the pigment of dietary cirrhosis of rats: its characteristics and its differentiation from hemofuscin. Amer J Path 20: 149–153, 1944
21. Wolman M: Lipid pigments, Handbuch der Histochemie. Vol V. Part II. Edited by W Graumann, K Neumann. Stuttgart, Gustav Fischer, 1964, pp 92–132
22. Hartroft WS, Porta EA: Ceroid. Amer J Med Sci 250:324–345, 1965

23. Hydén H, Lindstrom B: Microspectographic studies on the yellow pigment in nerve cells. Discuss Faraday Soc 9:436–441, 1950
24. Neglia W, Burrows L, Thompson SW, Schaffner F: Ultrastructural studies of hepatic pigment following administration of intravenous fat. Lab Invest 12:378–385, 1963
25. Terry RD, Sperry WM, Prodoff B: Adult lipidosis resembling Niemann-Pick's disease. Amer J Path 30:263–285, 1954
26. Landing BH, Shirkey HS: A syndrome of recurrent infection and infiltration of viscera by pigmented lipid histiocytes. Pediatrics 20:431–438, 1957
27. Oppenhemier EH, Andrews EC: Ceroid storage disease in childhood. Pediatrics 23:1091–1102, 1959
28. Winkler HH, Frame B, Saeed SM, Spindler AC, Brouillette JN: Ceroid storage disease complicated by rupture of the spleen. Amer J Med 46:297–301, 1969
29. Roberts WC, Levy RI, Fredrickson DS: Hyperlipoproteinemia: a review of the five types with first report of necropsy findings in type III. Arch Path 90:46–56, 1970
30. Daft FS, Sebrell WH, Lillie RD: Production and apparent prevention of a dietary liver cirrhosis in rats. Proc Soc Exp Biol Med 48:228–231, 1941
31. Yamori T, Mori Y: Fine structure of the reticuloendothelial system in the spleen with special reference to the red pulp. Tohoku J Exp Med 91:367–374, 1967
32. Simon GT, Burke JS: Electron microscopy of the spleen. III. Erythrophagocytosis. Amer J Path 58:451–470, 1970
33. Györkey F, Shimamura T, O'Neal RM: The fine structure of ceroid in human atheroma. J Histochem Cytochem 15:732–736, 1967
34. Fisher ER, Hellstrom HR: Ceroid-like colonic histiocytosis. Amer J Clin Path 42:581–593, 1964
35. Sjöstrand F: Über die Eigenfluoreszenz tierischer Gewebe mit besonderer Berücksichtigung der Saugetiere. Acta Anat: Suppl 1:1–136, 1945
36. Moore RD, Mumaw VR, Schoenberg MD: The structure of the spleen and its functional implications. Exp Molec Path 3:31–50, 1964
37. Weiss L: The structure of the normal spleen. Seminars Hemat 2:205–226, 1965
38. Burke JS, Simon GT: Electron microscopy of the spleen. I. Anatomy and microcirculation. Amer J Path 58:127–155, 1970
39. Roberts DK, Latta JS: Electron microscopic studies on the red pulp of the rabbit spleen. Anat Rec 148:81–101, 1964
40. Warren S, Root HF: Lipoid-containing cells in the spleen in diabetes with lipemia. Amer J Path 2:69–80, 1926
41. Tanaka Y, Brecher G, Fredrickson DS: Cellules de la maladie de Niemann-Pick et de quelques autres lipoïdoses. Nouv Rev Franc Hemat 3:5–16, 1963
42. Diezel PB: Lipidoses of the central nervous system, Modern Scientific Aspects of Neurology. Edited by JN Cummings. London, Edward Arnold, 1965, pp 98–145
43. Ferrans VJ, Hibbs RG, Burda CD: The heart in Fabry's disease: a histochemical and electron microscopic study. Amer J Cardiol 24:95–110, 1969
44. Abramov A, Schore S, Wolman M: Generalized xanthomatosis with calcified adrenals. Amer J Dis Child 91:282–286, 1956

45. Hoffman HN, Fredrickson DS: Tangier disease (familial high density lipoprotein deficiency): clinical and genetic features in two adults. Amer J Med 39:582–593, 1965
46. Hibbs RG, Ferrans VJ, Cipriano PR, Tardiff KJ: A histochemical and electron microscopic study of Gaucher cells. Arch Path 89:137–154, 1970
47. Schneck L, Volk BW, Saifer A: The gangliosidoses. Amer J Med 46:245–263, 1969
48. Suzuki K, Suzuki K, Chen GC: GM_1-gangliosidosis (generalized gangliosidosis): morphology and chemical pathology. Path Europ 3:389–408, 1968
49. Lough J, Fawcett L, Wiegensberg B: Wolman's disease. Arch Path 89: 103–110, 1970
50. Volk BW, Wallace BJ, Aronson SM: Some ultrastructural and histochemical aspects of lipidoses. Path Europ 3:200–217, 1968
51. Fredrickson DS: Classification and features of the lipidoses affecting the nervous system. Path Europ 3:121–142, 1968
52. Törö I, Ruzsa P, Röhlich P: Ultrastructure of early phagocytic stages in sinus endothelial and Kupffer cells of the liver. Exp Cell Res 26:601–603, 1962
53. Orci L, Pictet R, Rouiller C: Image ultrastructurale de pinocytose dans la cellule de Kupffer du foie de rat. J Microscopie 6:413–418, 1967
54. Matter A, Orci L, Forssmann WG, Rouiller C: The stereological analysis of the fine structure of the "micropinocytosis verminformis" in Kupffer cells of the rat. J Ultrastruct Res 23:272–279, 1968
55. Burke JS, Simon GT: Electron microscopy of the spleen. II. Phagocytosis of colloidal carbon. Amer J Path 58:157–181, 1970
56. Ashworth CT, Di Luzio NR, Riggi SJ: A morphologic study of the effect of reticuloendothelial stimulation upon hepatic removal of minute particles from the blood of rats. Exp Molec Path: Suppl 1:83–103, 1963
57. Hamilton RL: Ultrastructural aspects of hepatic lipoprotein synthesis and secretion, Proceedings of the 1968 Deuel Conference on Lipids on the Turnover of Lipids and Lipoproteins. Edited by G Cowgill, DL Estrich, PD Wood. Washington, DC, US Government Printing Office, 1969, pp 1–32
58. Hers HG: Inborn lysosomal diseases. Gastroenterology 48:625–633, 1965
59. Patrick AD, Lake BD: Deficiency of an acid lipase in Wolman's disease. Nature 222:1067–1068, 1969
60. Patrick AD, Lake BD: An acid lipase deficiency in Wolman's disease. Biochem J 112:29P, 1969

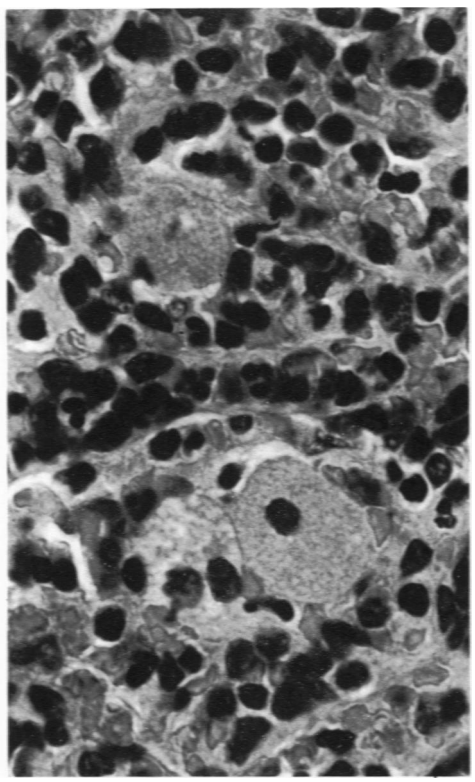
[*Illustrations follow*]

Fig 1–13 are light micrographs; Fig 14–30 are electron micrographs

Fig 1—Area of splenic red pulp showing foam cells and polymorphonuclear leukocytes (H&E, × 1000).

Fig 2—A group of foam cells, some of which contain ingested erythrocytes, are present in the red pulp (H&E, × 1000).

Fig 3—Semithin (0.5 μ) section of Maraglas-embedded tissue, showing three foam cells filled with cytoplasmic granules (alkaline toluidine blue, × 1500).



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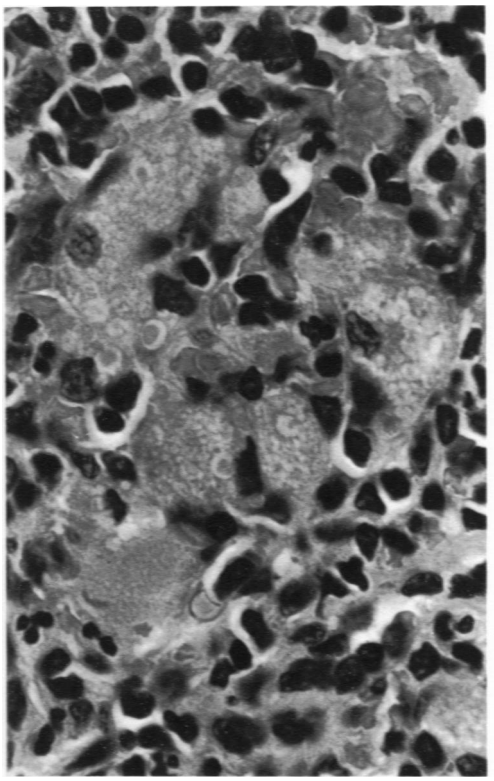

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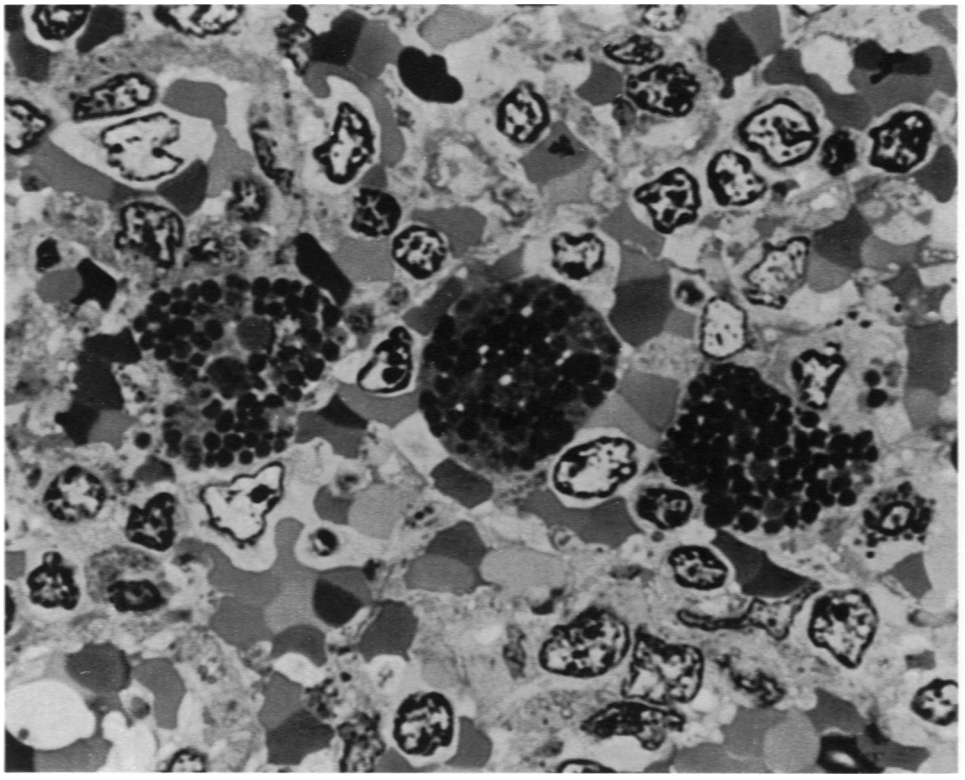

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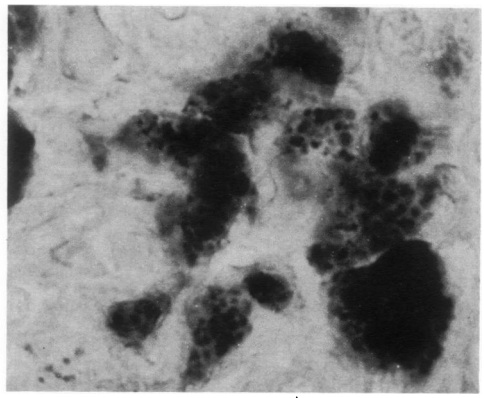
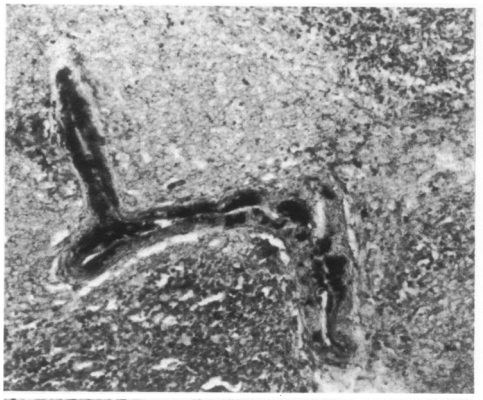
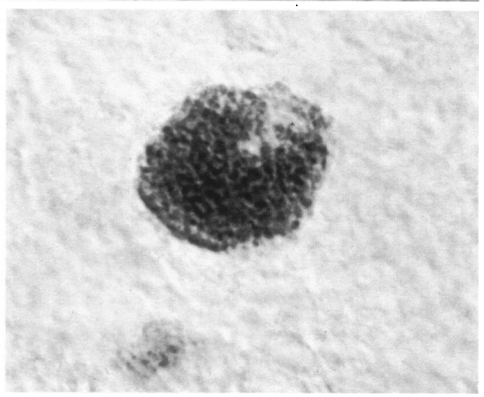
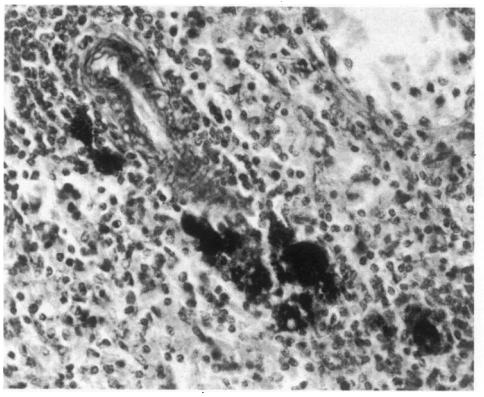
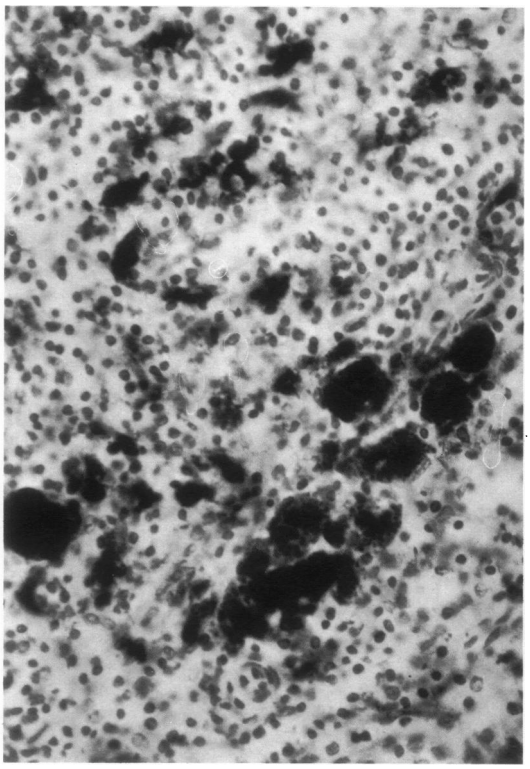










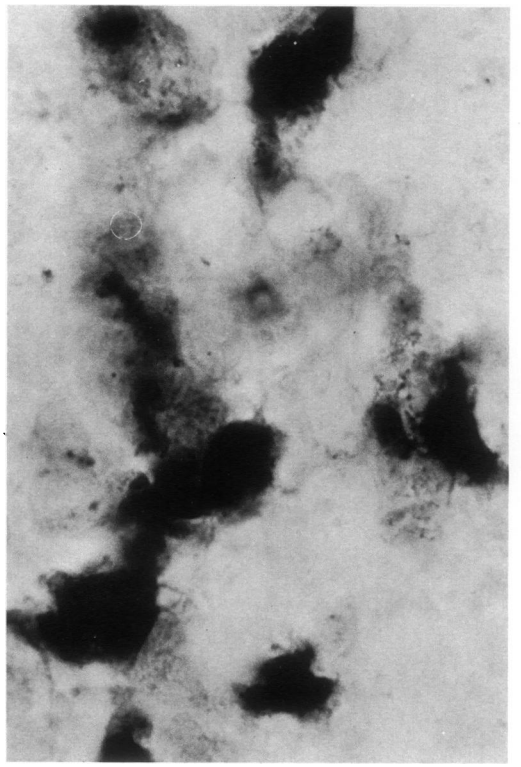
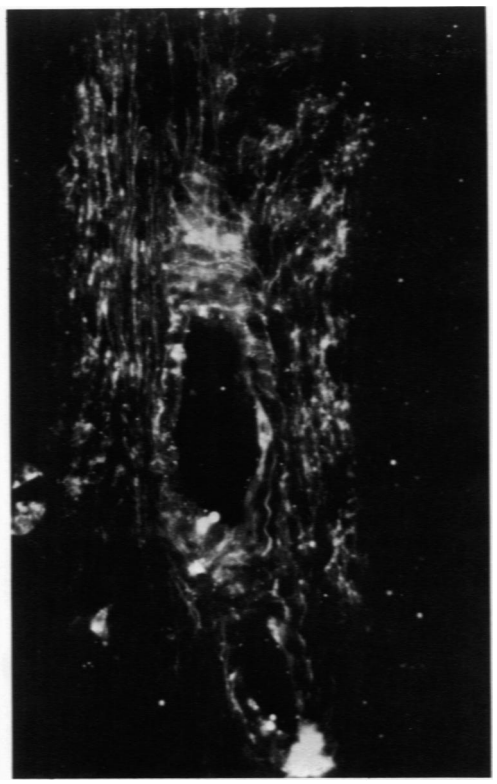


Fig 4—Granules in foam cells show intense staining with Sudan black B (× 1000). **Fig 5**—The walls of a blood vessel are infiltrated with lipid (Sudan black B stain, × 40). **Fig 6**—A foam cell stained by the Schultz method for cholesterol shows a moderately intense reaction (× 800). **Fig 7**—Several foam cells in the vicinity of a blood vessel show a positive periodic acid-Schiff reaction (× 150). **Fig 8**—Esterase preparation shows an intense reaction in mature foam cells and in smaller, irregularly shaped histiocytes (× 200). **Fig 9**—Acid phosphatase preparation shows a reaction pattern similar to that of the esterase reaction shown in Fig 8 (× 1000).



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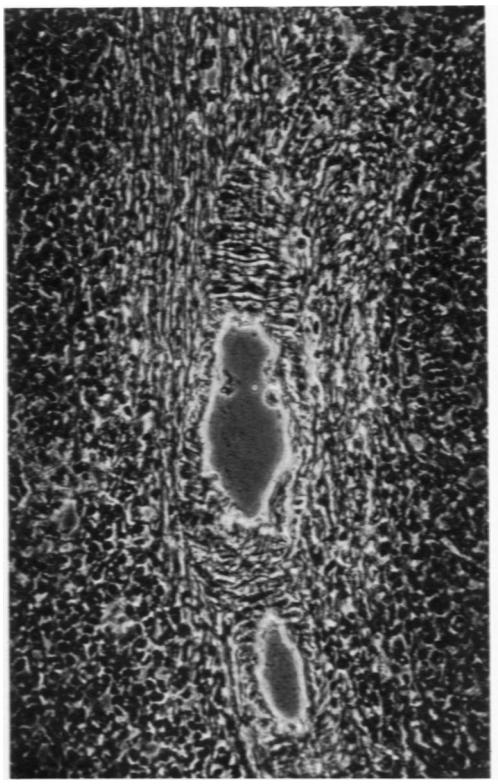

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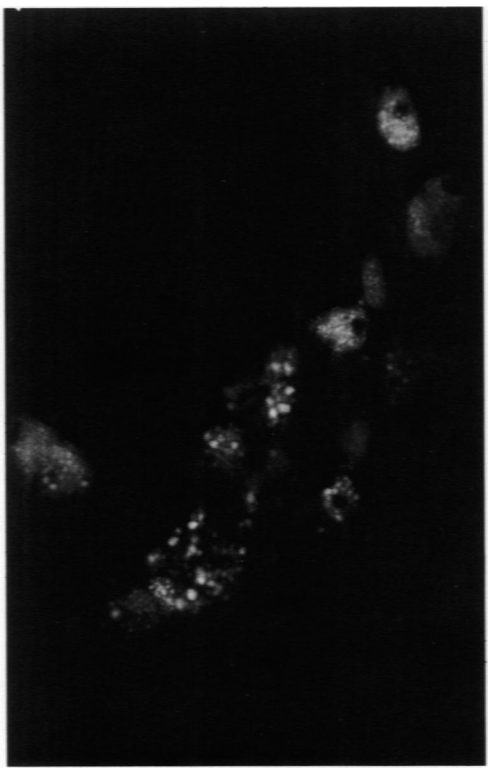

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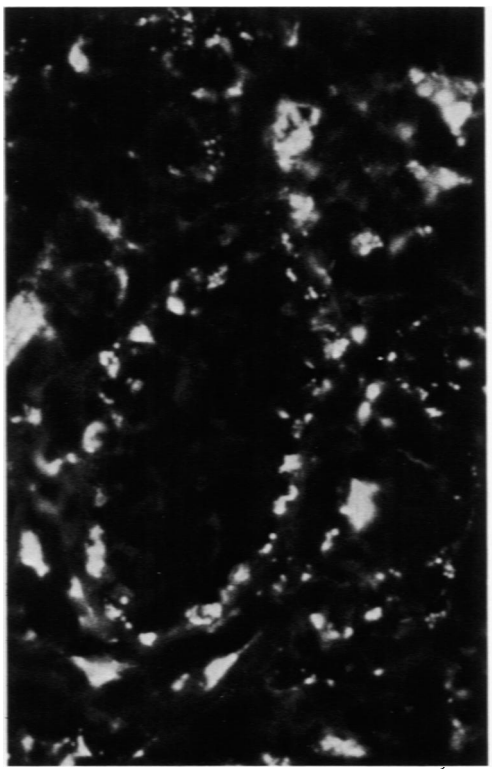

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Fig 10—Preparation stained with benzpyrene for the fluorescence microscopy of lipids shows the presence of lipid deposits in the vascular walls (× 200). **Fig 11**—Phase contrast view of same field shown in Fig 10, showing the structure of the blood vessel (× 200). **Fig 12**—Cytoplasmic granules in several foam cells show intense autofluorescence in an unstained section (× 400). **Fig 13**—Autofluorescent cytoplasmic granules are present in lining cells of a sinusoid and in surrounding foam cells in various stages of development (unstained section, × 1000).

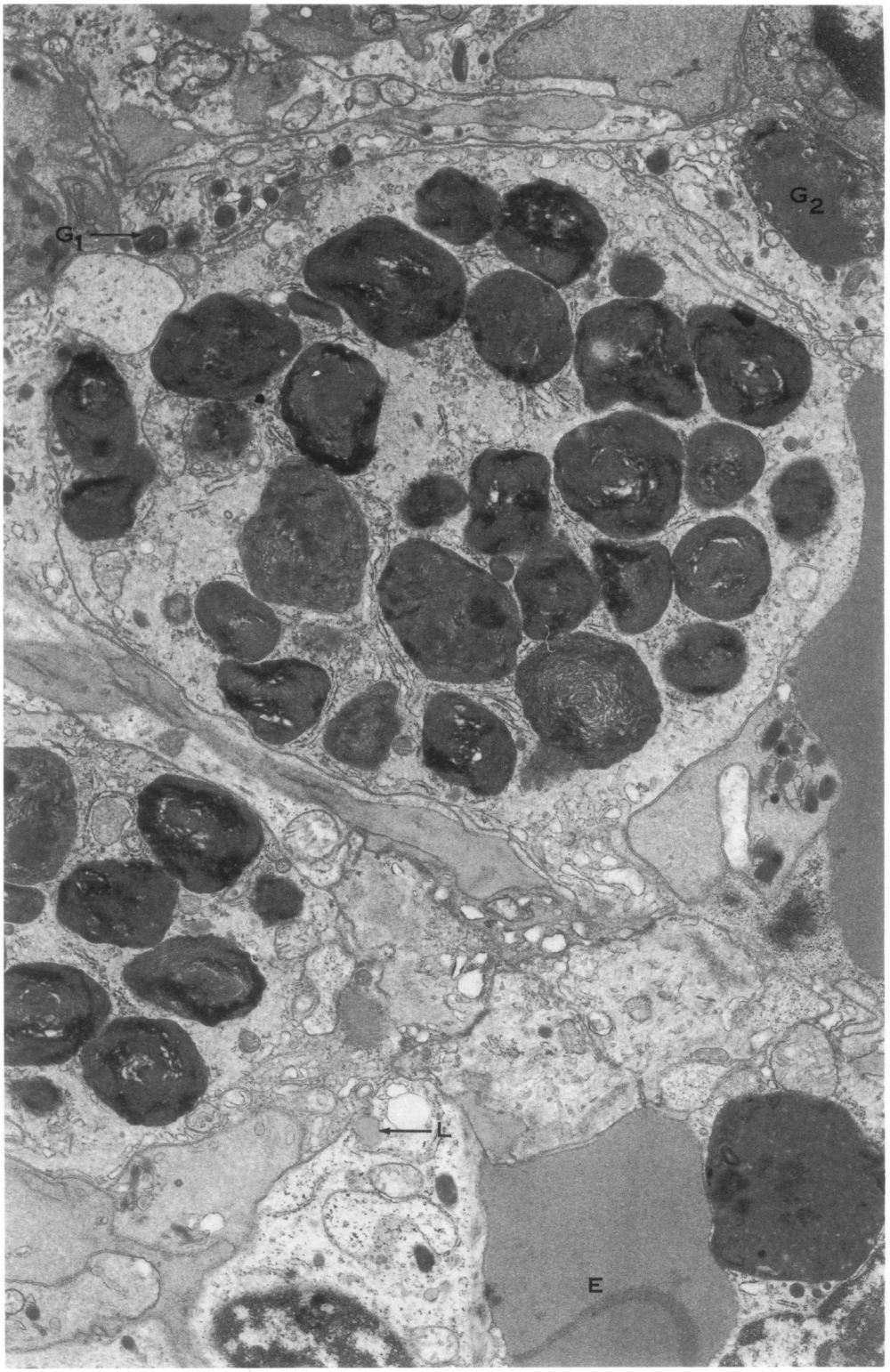

Fig 14—Two foam cells are present in the vicinity of a splenic sinusoid, the lumen of which is filled by an erythrocyte (*E*). The foam cells contain cytoplasmic filaments, a few cisterns of granular endoplasmic reticulum and numerous cytoplasmic granules composed of concentric lamellae. A lipid droplet (*L*) is present in the cytoplasm of a sinusoidal lining cell. An elongated cell process of a young histiocyte contains several cytoplasmic granules (G_1) in early stages of formation and a more mature granule (G_2) (× 11,600).

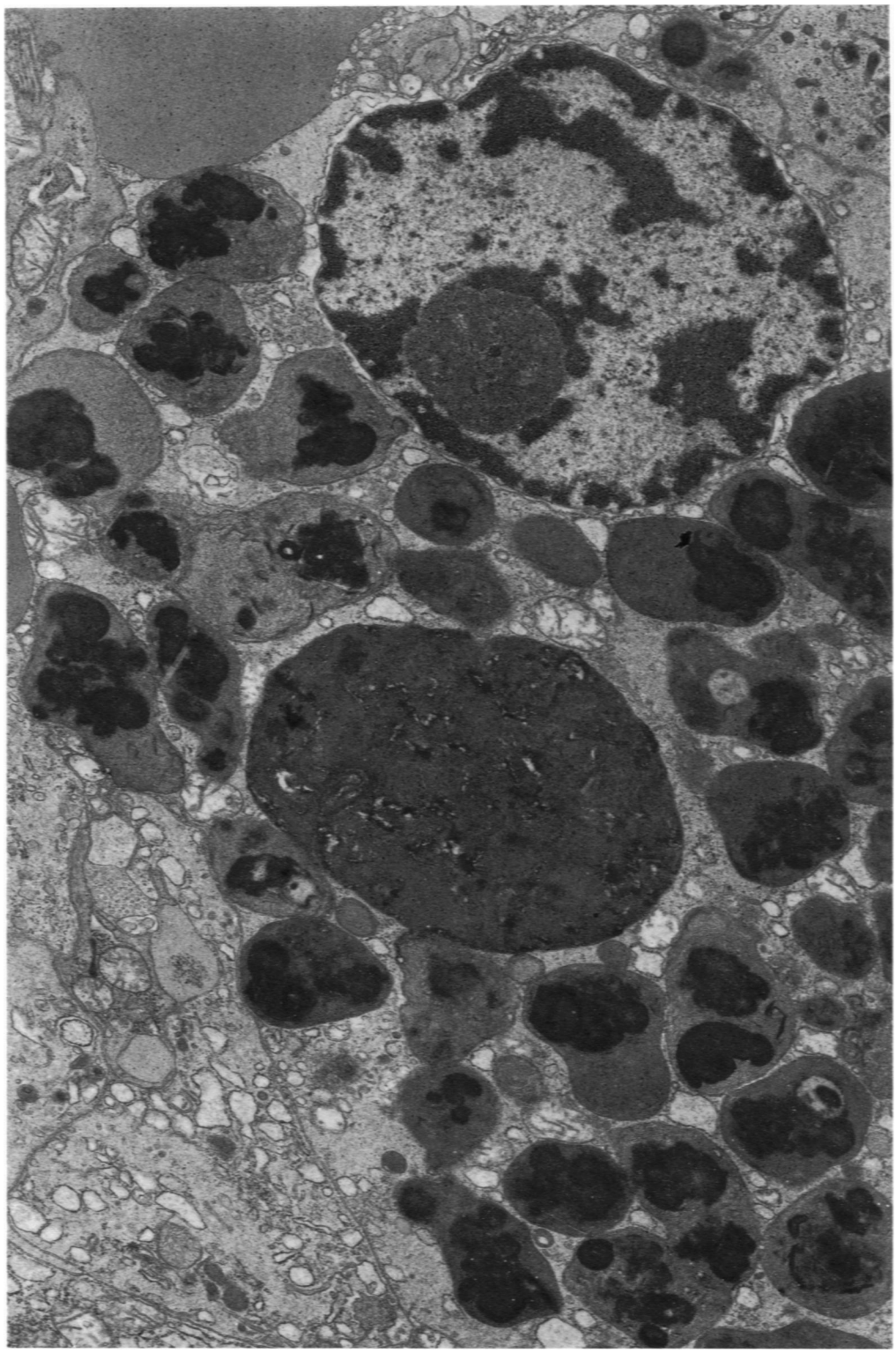

Fig 15—Portion of a foam cell showing the nucleus, nucleolus, a few mitochondria, scattered elements of granular endoplasmic reticulum and numerous cytoplasmic granules. In contrast to the more homogeneous granules shown in Fig. 14, most of the granules in this cell are composed of dense cores and lighter peripheral areas (× 23,750).

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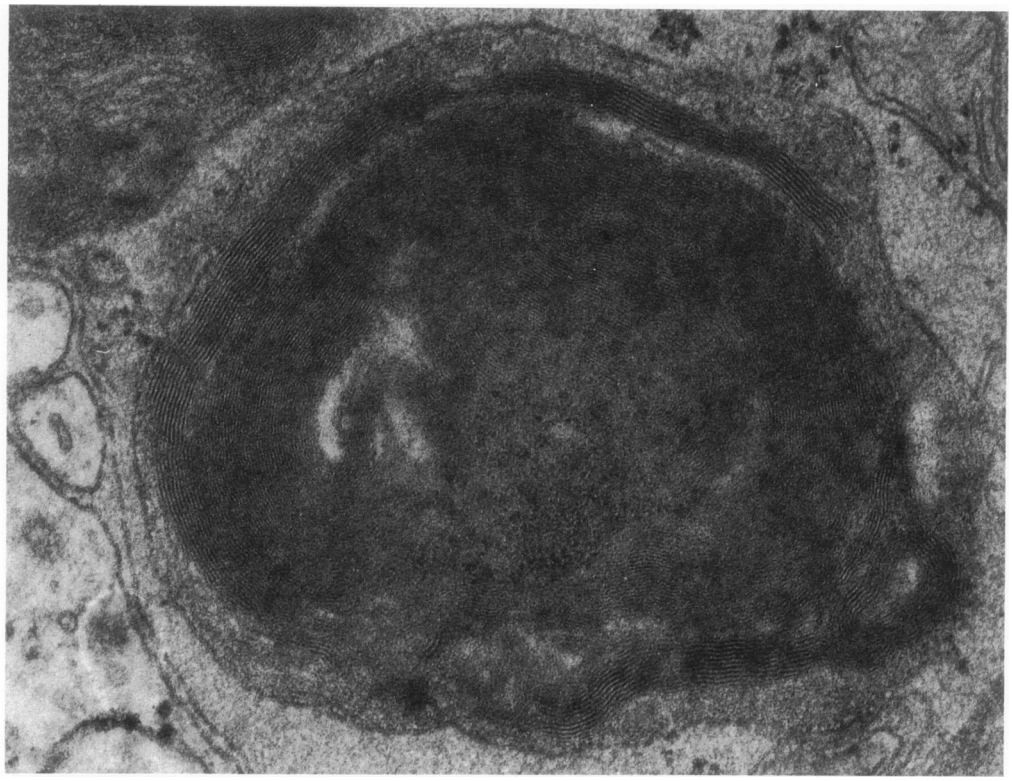

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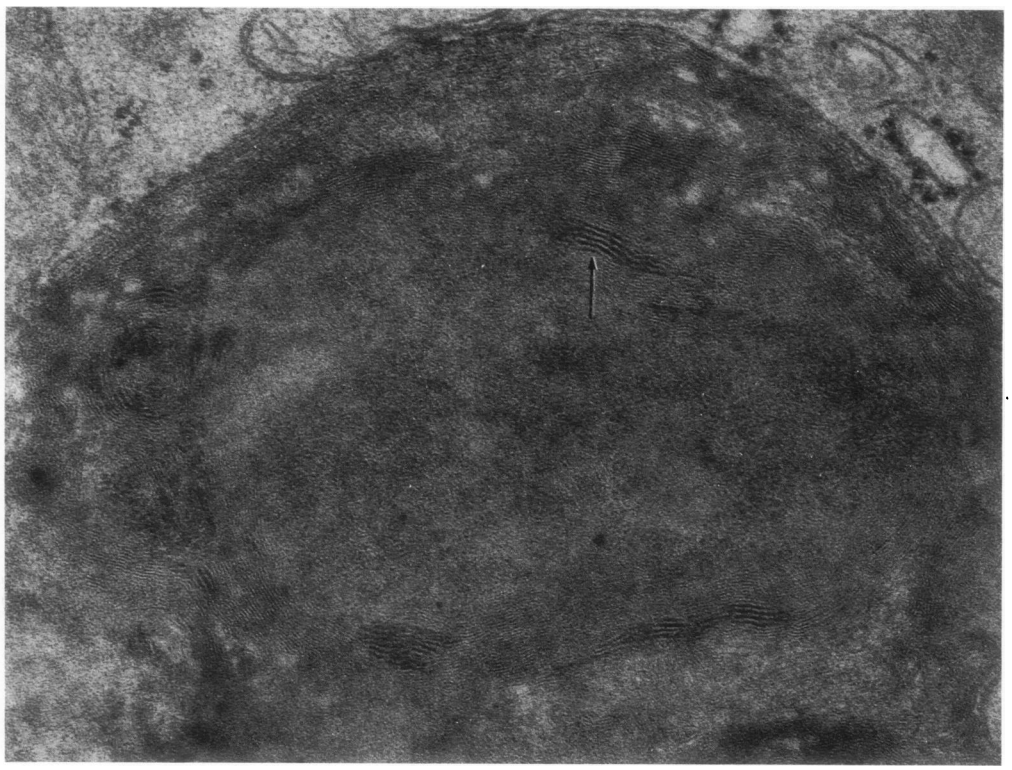

Fig 16—High-power view of cytoplasmic granule in foam cell. The granule is membrane-limited, has a thin rim of amorphous material at the periphery, and is composed of concentric, electron-dense lamellae. The periodicity of the lamellae is 40 A in the center, and 60 A in the periphery of the granule (× 90,000). **Fig 17**—Portion of a cytoplasmic granule composed of electron-dense, amorphous clumps of material, and several groups of concentric lamellae. Portions of some lamellae are thick and very electron-dense (*arrow*) (× 90,000).

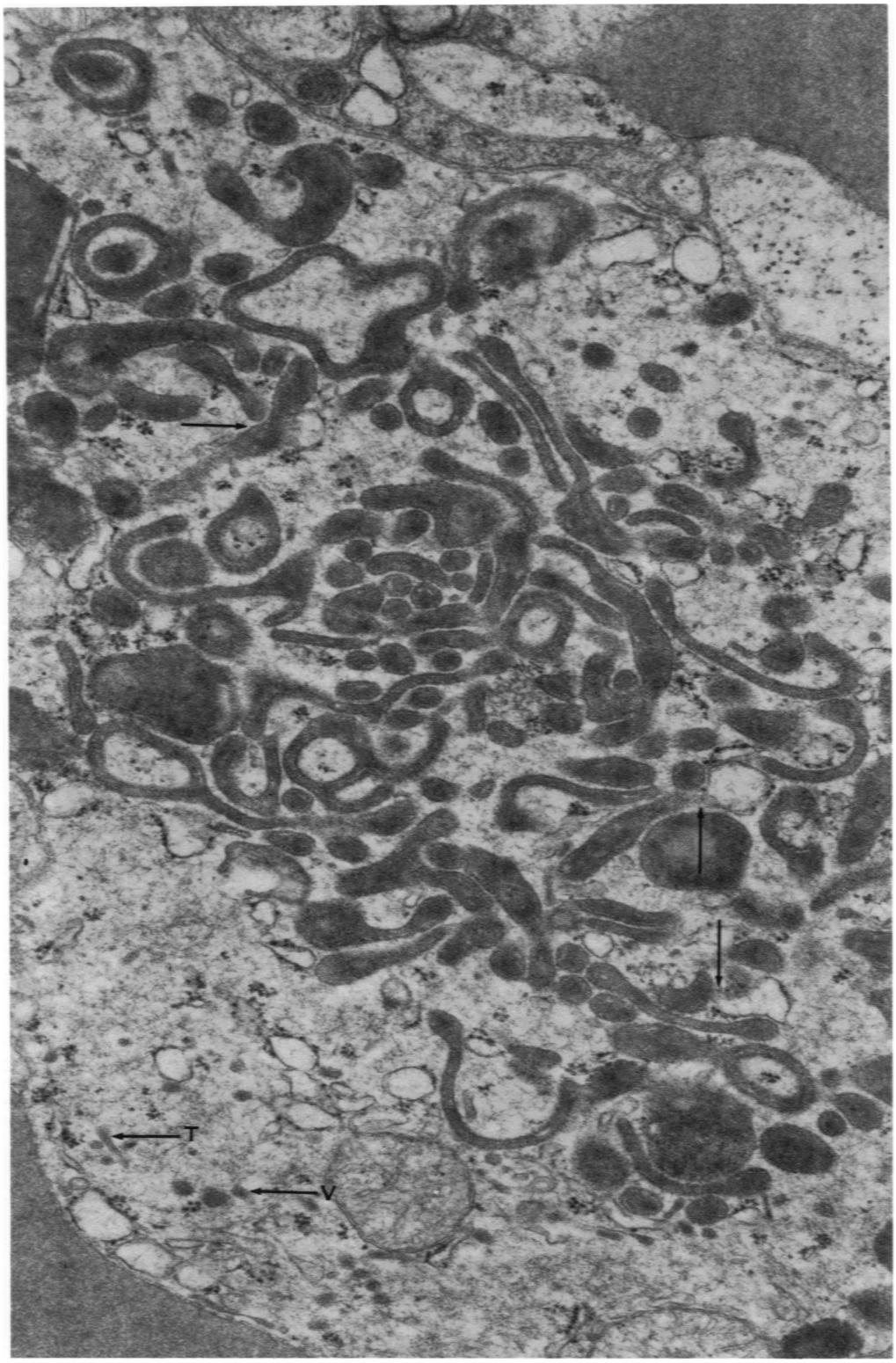

Fig 18—Portion of young histiocyte that contains numerous cisterns, tubules and vesicles filled with finely granular, electron-dense material. Several cisterns have knob-like dilatations at their ends. Some electron-dense cisterns are continuous (*arrows*) with areas of granular endoplasmic reticulum. Smaller tubules (*T*) and vesicles (*V*) are present near the surface of the cell (× 37,750).

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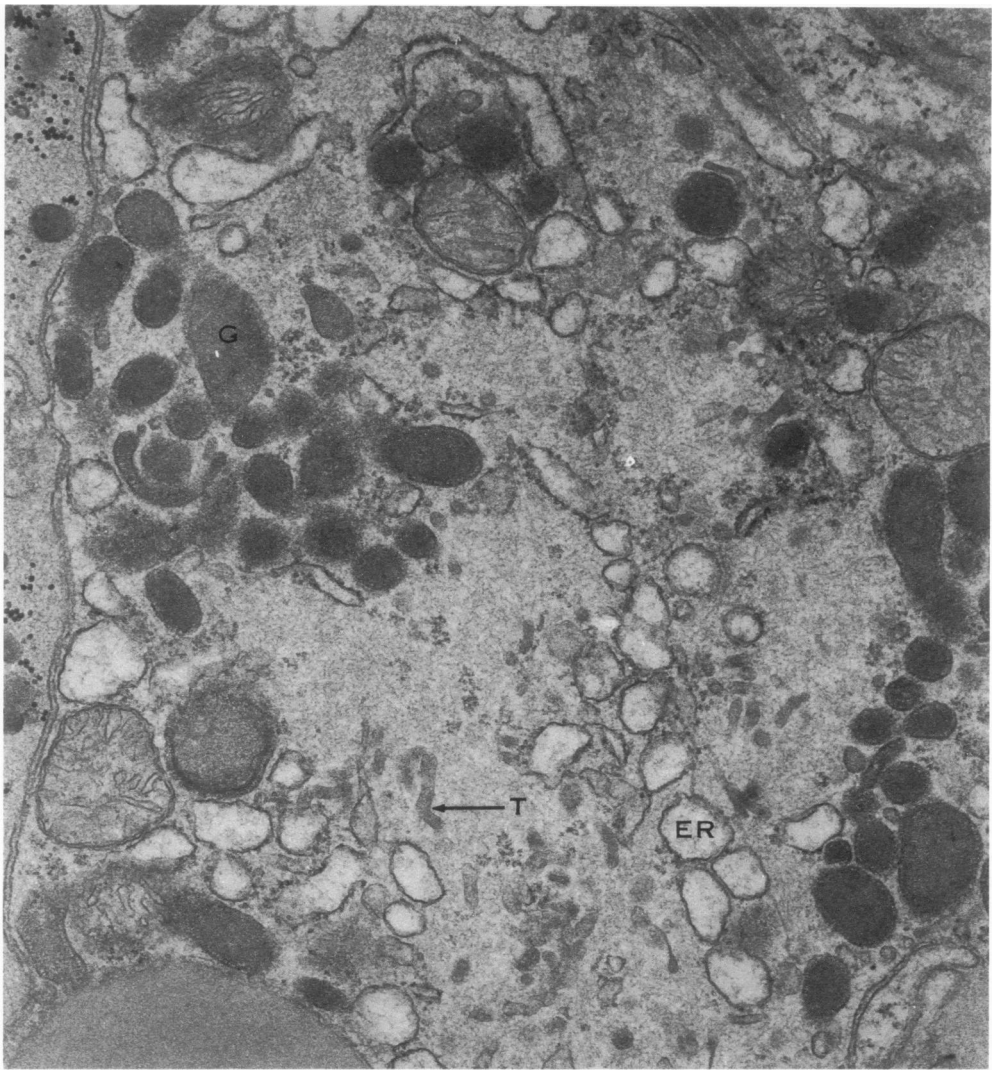

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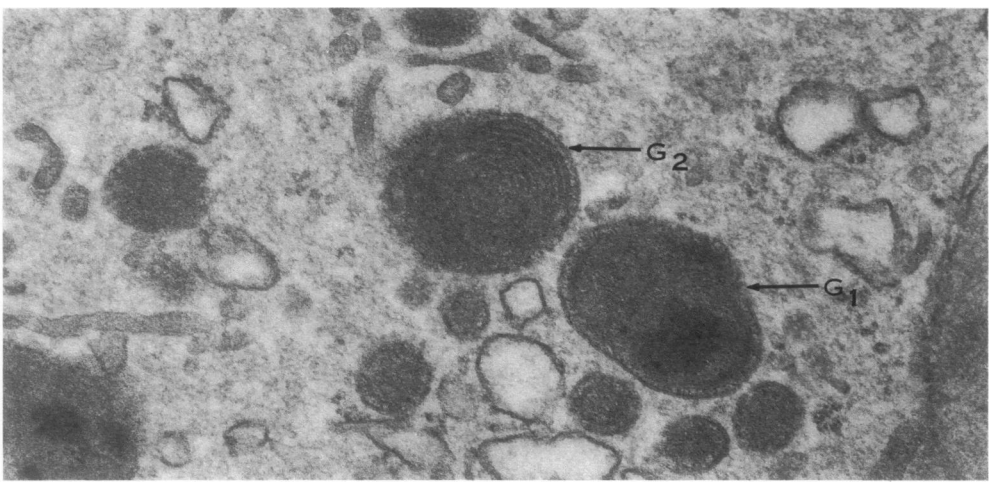
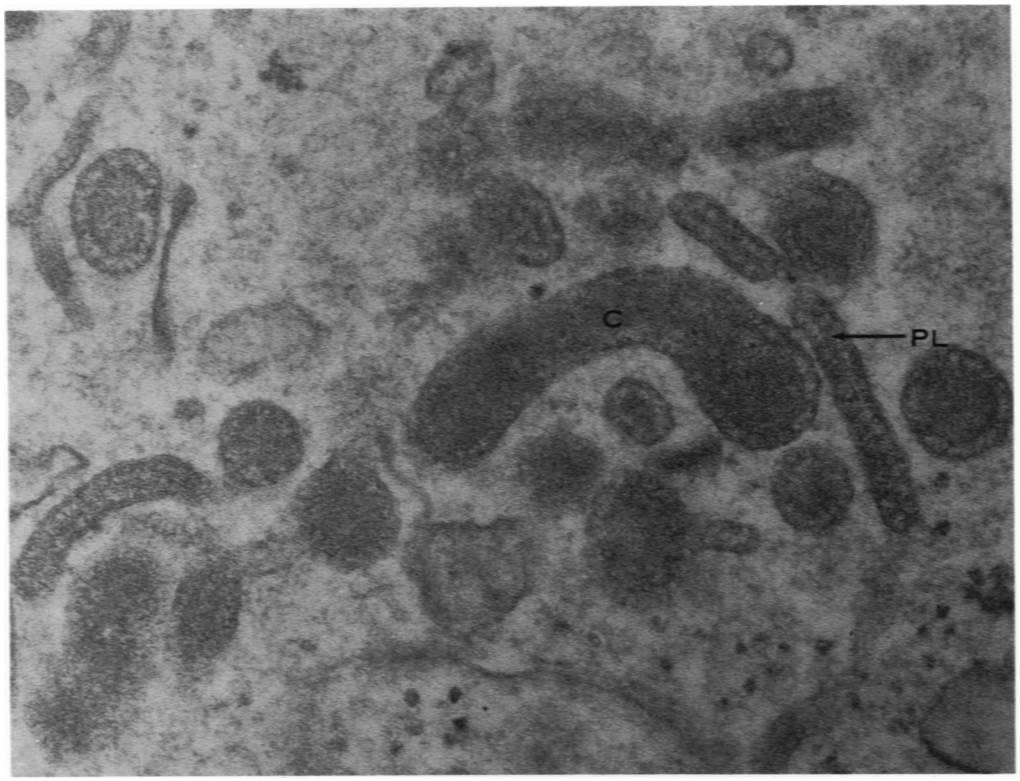


Fig 19—Electron-dense granules (*G*) in early stages of formation are present in the cytoplasm of a young histiocyte. This cell also contains less electron-dense, smooth-walled tubules (*T*) and a number of cisterns of granular endoplasmic reticulum, some of which (*ER*) are partially denuded of ribosomes (× 30,250). **Fig 20**—High-power view of cytoplasmic granules in early stages of formation. The granules are limited by a single, trilaminar membrane. One granule (G_1) contains unorganized, electron-dense material and a few concentric, paired lamellae. Another granule (G_2) is composed of more fully organized concentric lamellae. A zone of decreased electron density is present between the limiting membrane and the outermost concentric lamellae (× 67,500).



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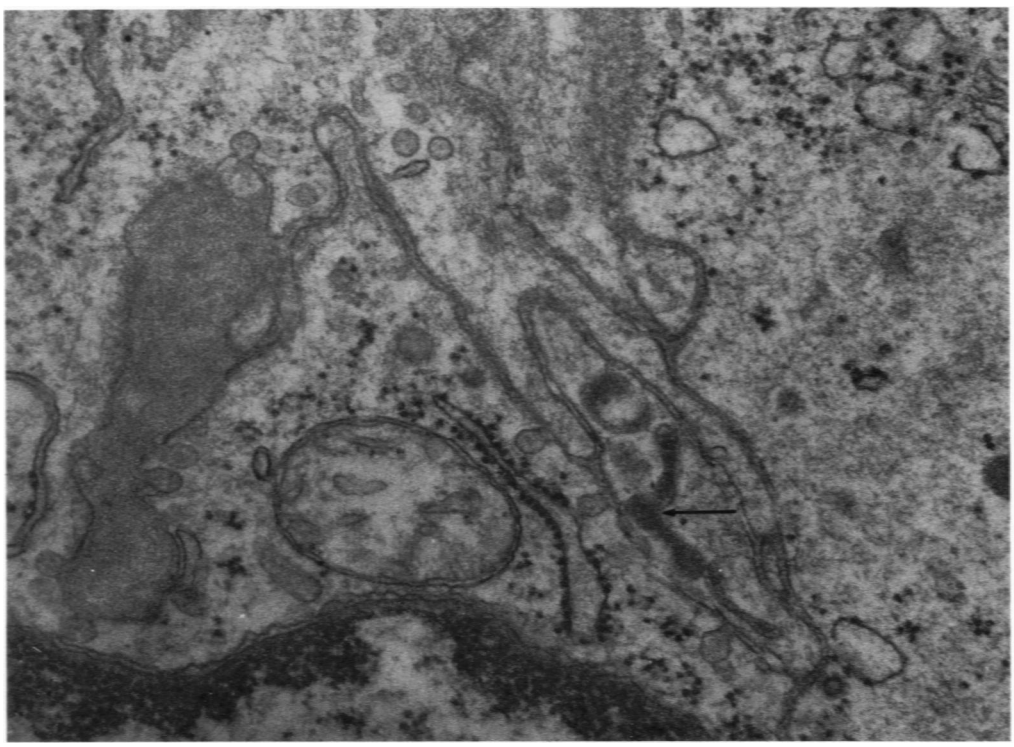

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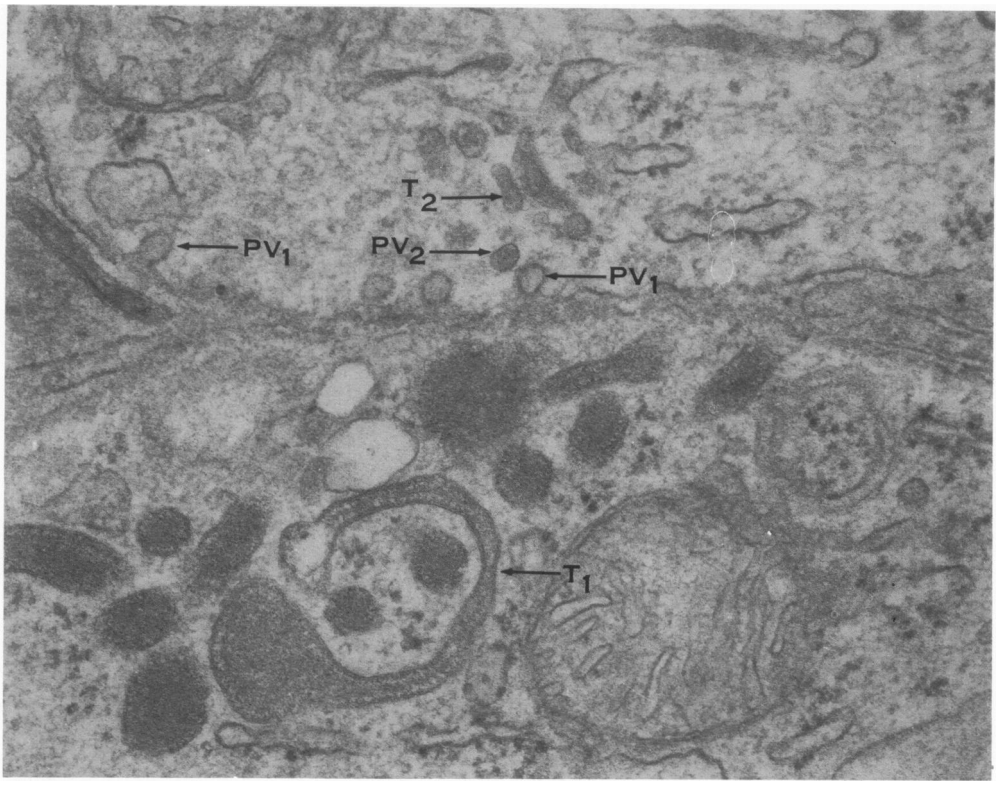
Fig 21—High-power view of electron-dense cisterns in the cytoplasm of a young histiocyte. Pairs of lamellae (*PL*) are present within several cisterns and tubules and are surrounded by peripheral clear zones. One cistern (*C*) contains less well organized material (× 86,000).
Fig 22—High-power view of portions of cell surfaces of young histiocytes. The plasma membranes have extensive infoldings associated with numerous pinocytotic vesicles. Electron-dense material, presumed to be lipid, is present in one infolding (*arrow*) (× 48,700).



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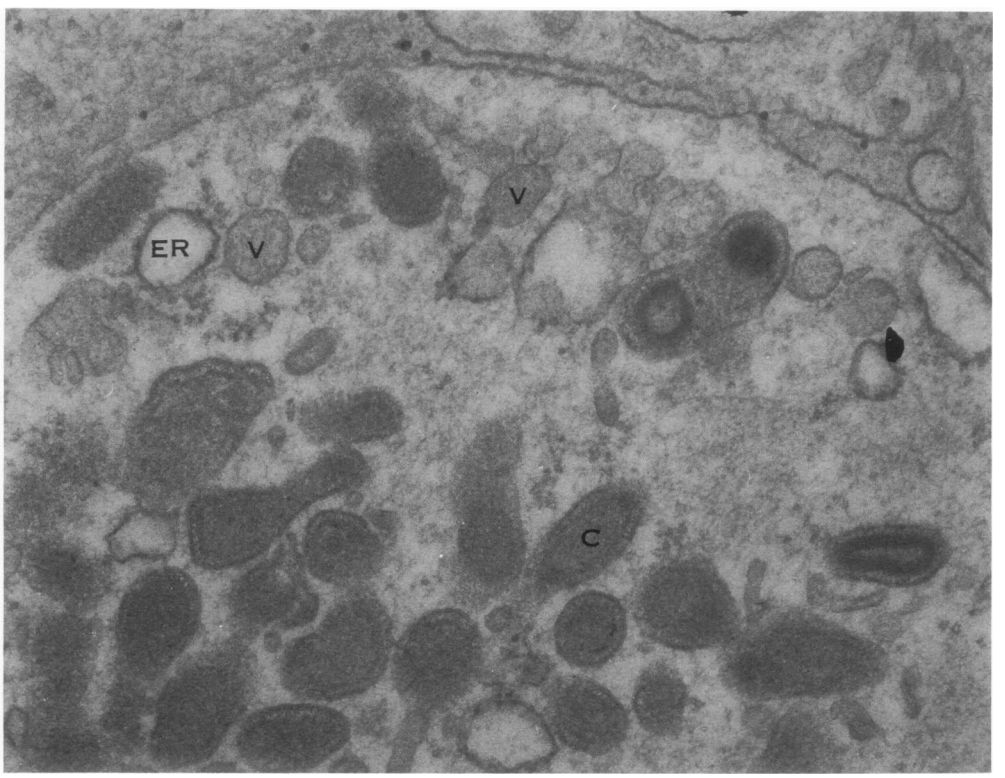

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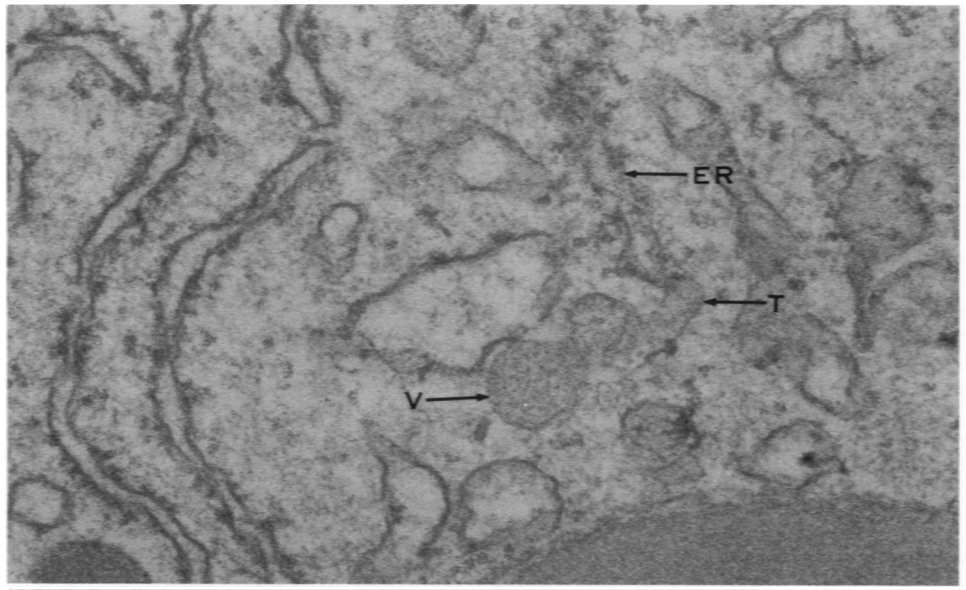
Fig 23—Portion of the cytoplasm of two adjacent young histiocytes. One cell contains several electron-dense tubules, one of which (T_1) has a large dilatation and contains lamellar material. Several pinocytotic vesicles (PV_1) are budding off the plasma membrane of the other cell. Other pinocytotic vesicles (PV_2) and tubules (T_2) filled with electron-dense material, are present in the adjacent cytoplasm (× 70,150). **Fig 24**—Area of cytoplasm near the plasma membrane of a young histiocyte showing several electron-dense cisterns (*C*) with partially organized material, and numerous smooth-walled vesicles (*V*). Note the difference in thickness of the limiting membranes of these two types of structures. Elements of granular endoplasmic reticulum (*ER*) also are present (× 69,750).



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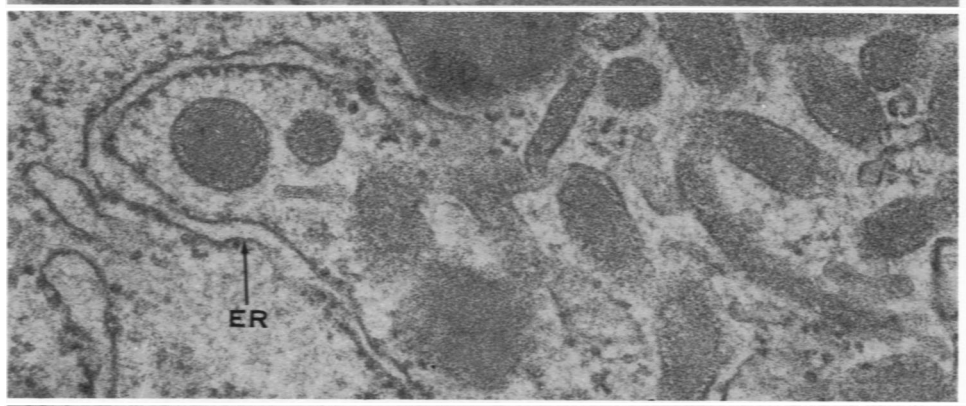

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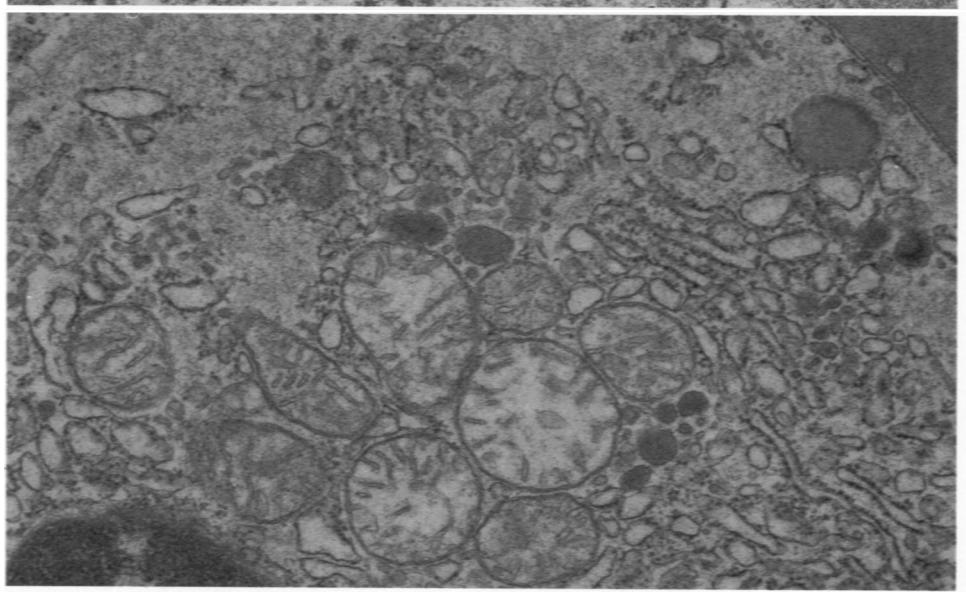

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Fig 25—Area of granular endoplasmic reticulum (*ER*) in young histiocyte shows continuity with a dilated, smooth-walled tubule (*T*) and a vesicle (*V*), which contain moderately electron-dense material (× 84,500). **Fig 26**—Areas of granular endoplasmic reticulum (*ER*) in young histiocyte show continuity with tubules filled with electron-dense material (× 74,500). **Fig 27**—View of a young histiocyte showing part of the nucleus, several mitochondria, numerous cisterns of granular and agranular endoplasmic reticulum, cytoplasmic filaments, and a few, small dense bodies that may represent granules in early stages of formation. A lipid droplet is free in the cytoplasm near the cell membrane (× 22,000).

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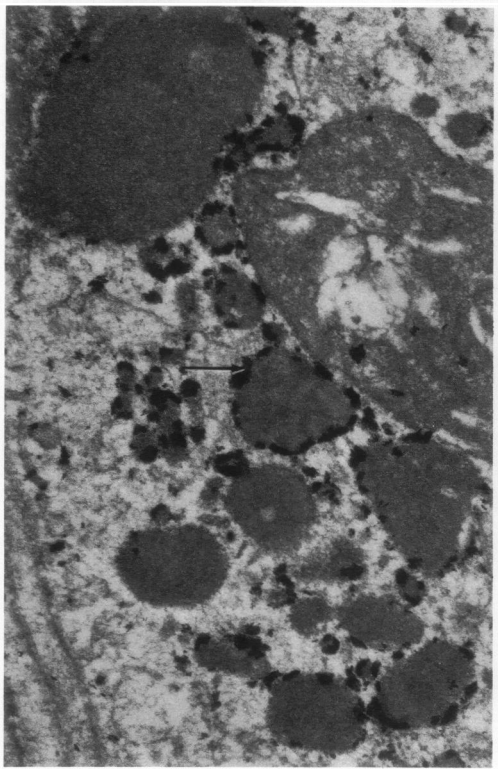

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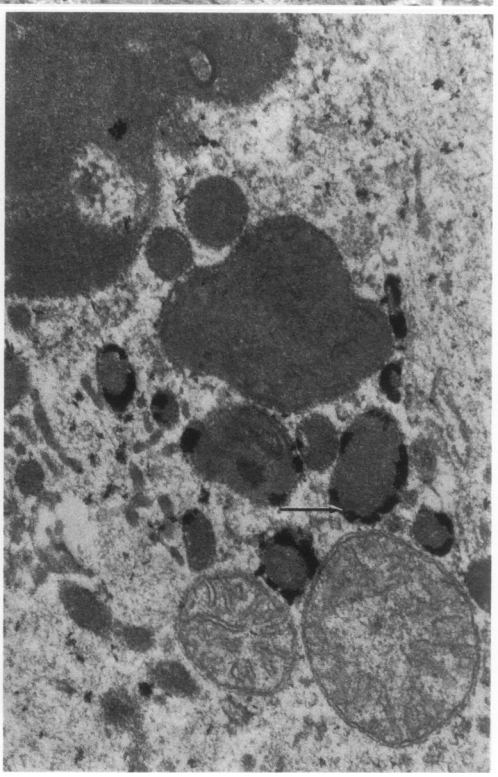

Fig 28—Clusters of lipid droplets (*arrows*) are present in the basement membranes of a small blood vessel (× 10,580). **Fig 29 and 30**—Preparations incubated for the demonstration of acid phosphatase activity. Reaction product is present on the membranes of developing cytoplasmic granules in young histiocytes (Fig 29, × 37,750; Fig 30, × 31,900).